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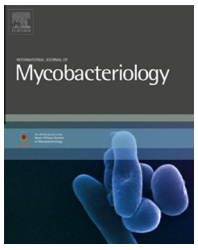

The role of porins in copper acquisition by mycobacteria

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ABSTRACT

Aims and objectives: Copper poisoning in macrophages plays an important role in immunity against invading pathogens. Many bacteria, including *Mycobacterium tuberculosis* (*Mtb*), have evolved mechanisms to combat copper-derived innate immune responses. Copper homeostasis in *Mtb* consists of several components, including a multi-copper oxidase, copper efflux pump, cytoplasmic metallothionein and copper-sensing transcriptional regulators. However, components involved in copper uptake are unknown, which prompted this study to investigate the possible role of porins in copper uptake in mycobacteria.

Methods: *Mycobacterium smegmatis* porin mutants were created and tested for their ability to grow under copper-reduced or copper-rich conditions. The *M. smegmatis* porin gene *mspA* was expressed in *Mtb*, and its copper susceptibility profile was investigated in the presence of different copper concentrations. The expression level of a copper detoxifying protein, mycobacterial multi-copper oxidase (MmcO), was monitored by western blot to assess intracellular copper content.

Results: Deletion of porin genes from *M. smegmatis* caused a severe growth defect on trace copper medium. Copper supplementation alleviated this phenotype. The inability to acquire copper in sufficient amounts due to lack of porins can explain this phenomenon. Moreover, porin mutants showed elevated tolerance to copper at concentrations that were toxic for wild-type strains, indicating that the lack of porins protects these strains from copper poisoning. On the other hand, heterologous expression of *mspA* in *Mtb* significantly impaired growth at 2.5 μM copper and eliminated growth at 15 μM, while wild-type *Mtb* eventually reached its normal cell density at this copper concentration. Consistent with a role of porins in copper uptake, expression levels of MmcO in *Mtb* expressing the *M. smegmatis* porin *mspA* was above wild-type levels, indicating that cytoplasmic copper-sensing transcriptional regulators respond by derepressing the expression of copper resistance genes. Moreover, the polyamine spermine, a known inhibitor of porin activity in gram-negative bacteria, increased the tolerance of wild-type *Mtb* for copper suggesting that endogenous outer membrane proteins with channel-forming activity exist and contribute to copper acquisition and toxicity in *Mtb*.

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Conclusions: It was concluded from these results that porins are involved in copper uptake in mycobacteria. Moreover, the outer membrane of *Mtb* was found to be an important barrier against copper intoxication so that permeabilization of this barrier (*e.g.*, by porins) renders *Mtb* extremely vulnerable to copper. Consequently, copper homeostasis of *Mtb* provides a promising drug target for the development of a new class of anti-tuberculosis compounds that can induce a copper hypersensitivity phenotype in *Mtb*.